EFFECT OF BASE TILTING ON THE OPTICAL ACTIVITY OF NUCLEIC ACIDS: A HYPOTHESIS*

Jen Tsi Yang and Tatsuya Samejima

Cardiovascular Research Institute and Department of Biochemistry, University of California San Francisco Medical Center, San Francisco, California 94122 and Department of Chemistry, Aoyama Gakuin University, Tokyo

Received October 21, 1968

DNA, RNA, and synthetic polynucleotides all display multiple Cotton effects with three maxima (peaks) and two minima (troughs) between 190 and 300 mμ. Figures 1A and 1B illustrate the ORD[1] of a DNA (Samejima and Yang, 1965) and double-stranded RNA (Samejima et al., 1968). One striking difference in the ORD between DNA and RNA was first reported by Samejima and Yang (1964, 1965): the second maximum (near 230 mμ) of DNA is always much larger than the first one at 290 mμ, whereas the opposite is true for RNA which has a rotation close to zero for its second maximum. This observation is also supported by the corresponding CD (Brahms and Mommaerts, 1964) and illustrated in Figs. 2A and 2B. DNA has two almost equally intense CD bands, one positive on the long wavelength side and the other negative (the conservative type over the wavelength range studied according to Bush and Brahms, 1967), but the large positive band of RNA is followed by an extremely small negative one on the short wavelength side (the non-conservative type). Both DNA and RNA also show a small negative CD band around 300 mμ (Sarkar et al., 1967).

---

* This work was supported by USPHS grants (GM-K3-3441, GM-10880, and HE-06285).

[1] Abbreviations: ORD, optical rotatory dispersion; CD, circular dichroism.

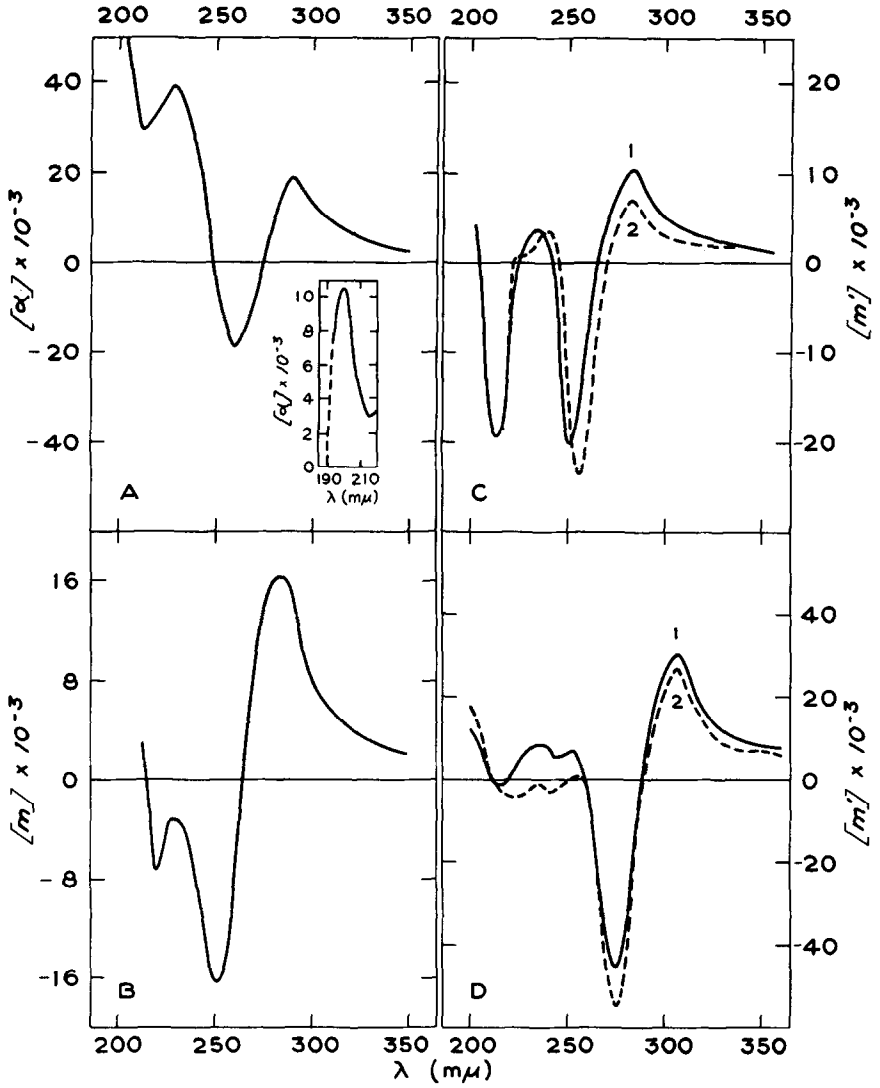

Fig. 1. ORD of nucleic acids and polynucleotides. (A) Specific rotation of salmon sperm DNA in 0.15 M KF at 27°C (Samejima and Yang, 1965). (B) Mean residue rotation of rice dwarf virus RNA in 0.01 M SSC at 24°C (Samejima et al., 1968). (C) Reduced mean residue rotation of (1) $(dA)_n.(rU)_n$ and (2) $(rA)_n.(dT)_n$ in 0.05 M $NaClO_4$ at 20°C (Ts'o et al., 1966). (D) Reduced mean residue rotation of (1) $(dC)_n$ and (2) $(rC)_n$ in 0.05 M $NaClO_4$ and 0.001 M CH_3 COONa at 20°C (Ts'o et al., 1966).

Since a single-stranded RNA molecule may adopt hairpin-like or loop-like conformations unlike the regular double-stranded DNA helix, the observed difference in the ORD and CD spectra might be attributed to this difference. This possibility, however, is ruled out by the fact that the

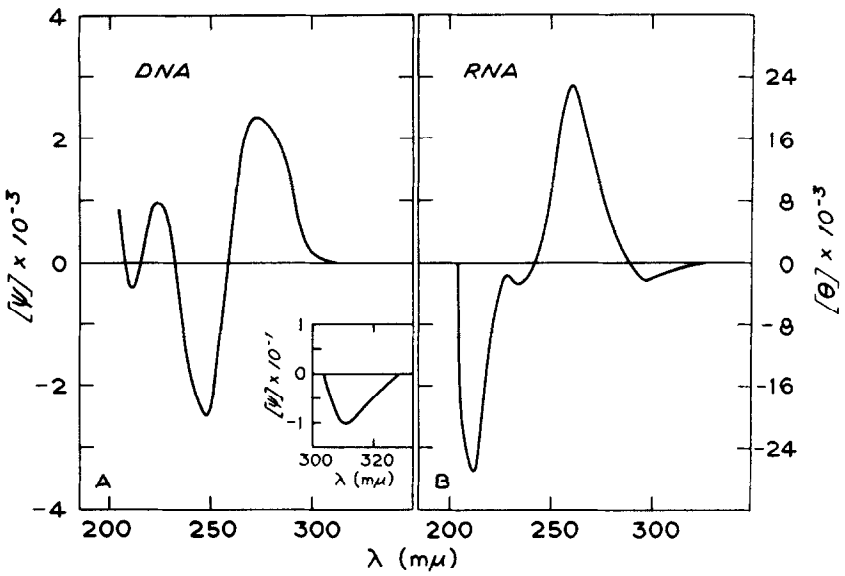

Fig. 2. CD of nucleic acids. (A) Specific ellipticity of salmon sperm DNA in 0.005 M tris, 0.0005 M Mg^{2+}, and 0.1 M NaCl at 25°C (Sarkar and Yang, 1967). (B) Mean residue ellipticity of rice dwarf virus RNA in 0.01 M SSC at 24°C (Samejima et al., 1968).

---

double-stranded RNA's such as rice dwarf virus RNA (Sato et al., 1966; Samejima et al., 1968) have the same ORD and CD profiles as the single-stranded ones (see Figs. 1B and 2B).

The major difference in chemical character of DNA and RNA is the constituent pentose (and of course the difference between bases thymine and uracil). The Cotton effects of monoribonucleotides and monodeoxyribonucleotides for each purine or pyrimidine base are, however, very similar, even though ribose has one more asymmetric carbon than deoxyribose (Yang et al., 1966). Furthermore, the shape of the ORD for polydeoxycytidylic acid, $(dC)_n$, is very similar to that for polyribocytidylic acid, $(rC)_n$ (see Fig. 1D), as well as for polydeoxythymidylic and polyribouridylic acids (Ts'o et al., 1966). We thus believe that it is an alteration in the geometric arrangement of the polynucleotide chains and conformation of the nucleic acids rather than simply introduction of another asymmetric center at the 2'-carbon atom of the pentose which gives rise to the

observed difference in the Cotton effects of DNA and RNA.

It is instructive to consider various structures of the double-stranded nucleic acids. DNA can exist in three forms (A, B, and C) which give different X-ray diffraction patterns (Feughelman et al., 1955; Wilkins, 1957; Langridge et al., 1957). The B form having the planes of the paired bases virtually perpendicular to the helical axis exists in dilute aqueous solution. On the other hand, the A form that prevails in dried fibers at low relative humidity (less than 75%) has the planes of paired bases tilted by about 20°. The pentose rings are situated radially from the helical axis in the B form, but incorporated into the main helical chain in the A form. The distances between the helical axis and the base pairs also differ in the two forms. Thus, their mode of stacking is quite different (see, for example, Tsuboi and Higuchi, 1968). Comparison of atomic models of double-stranded DNA with double-stranded RNA or DNA-RNA hybrids is even more illuminating. A double-stranded RNA or DNA-RNA hybrid molecule cannot have a structure similar to the B form of DNA because of the steric hindrance due to the presence of the 2'-hydroxyl groups. Rather, the successive bases of the polynucleotide chains in these two cases must be tilted (or even twisted) with respect to the helical axis resulting in formation of a double helix very similar to the A form of DNA. (For a lucid discussion of these models, see Tsuboi and Higuchi, 1968.) We therefore believe that the mode of base stacking, the orientation of the pentose rings, and the orientation of the base pairs relative to the helical axis are responsible for the observed difference in ORD and CD of nucleic acids.

We propose the following working hypothesis for the optical activity of polynucleotides as well as nucleic acids in aqueous solution:

(a) The presence or absence of the 2'-hydroxyl groups on the pentose rings primarily influences the geometry of the stacked bases in nucleic acids. This in turn determines the relative magnitudes of the two ORD

maxima and intensities of the positive and negative CD bands clustered around the 260-mμ absorption band. (The only known exception is polyinosinic acid which has an inverse ORD profile; see, Sarkar and Yang, 1965b.)

(b) The stacking of bases perpendicular to the helical axis leads to a larger second ORD maximum (near 230 mμ) than the first maximum (at 290 mμ) and two almost equal but opposite CD bands as in DNA. Tilting of the bases reduces the second and increases the first ORD maximum and decreases the negative and increases the positive CD band as in RNA. (The small negative CD band near 300 mμ is not under consideration here.)

(c) Polydeoxyribonucleotides can have stacked bases perpendicular or tilted to the helical axis (see Figs. IA and ID), whereas polyribonucleotides or DNA-RNA hybrids always have tilted bases (see Figs. IC and ID). (We further speculate that these rules apply to single-stranded as well as double-stranded polynucleotides.)

Brahms and Mommaerts (1964) studied the CD of DNA in 80% ethanol at low salt concentration and reported an intermediate form between 40 and 55°C, which differs from both native and completely denatured DNA. These authors' results were very similar to the CD spectrum of RNA; the intensity of the positive band on the long wavelength side was about three times that of native DNA and the negative band was almost completely destroyed. It is highly suggestive that this intermediate form of DNA has tilted bases instead of bases perpendicular to the helical axis. Our working hypothesis also predicts that the A form of DNA would have its ORD and CD spectra resembling those of RNA. Preliminary study of the DNA films showed that the "moistened" film retained the same CD profile as that in solution and the dried film had markedly different profile. But reproducible quantitative results are still not realized because of experimental difficulties in the preparation of a uniform, unoriented film under low relative humidity.

The multiple Cotton effects of nucleic acids and polynucleotides are generally explained in terms of base stacking (Tinoco, et al., 1963; Bradley et al., 1963). Thus, their magnitude decreases with unstacking of the bases which occurs at elevated temperatures. (However, protonation or deprotonation of the bases can change the Cotton effects, even though the stacking may remain unchanged; see Sarkar and Yang, 1965c.) More recently, the formation of base pairings in RNA and polyribonucleotides was found to cause a blue shift of the ORD and CD spectra (Sarkar and Yang, 1965a; Cantor et al., 1967; Hashizume and Imahori, 1967), thus providing a new means for studying the RNA conformation. This communication emphasizes a third finding that the relative magnitudes of the ORD maxima and the relative intensities of the CD bands are closely related to the geometry of stacked bases and thereby the conformation of the nucleic acids.

Acknowledgments. - We are indebted to Professor Masamichi Tsuboi for calling to our attention the models of various double-stranded nucleic acids and to Professor Leonard Peller for his critical comments. Excellent technical assistance of Miss Helen Gee is gratefully acknowledged.

References

Bradley, D. F., Tinoco, I., Jr., and Woody, R. W., Biopolymers 1, 239 (1963).
Brahms, J. and Mommaerts, W. F. H. M., J. Mol. Biol. 10, 73 (1964).
Bush, C. A. and Brahms, J., J. Chem. Phys. 46, 79 (1967).
Cantor, C. R., Jaskunas, S. R., and Tinoco, I., Jr., J. Mol. Biol. 20, 39 (1966).
Feughelman, M., Langridge, R., Seeds, W., Stokes, A., Wilson, H., Hooper, C., Wilkins, M., Barclay, R., and Hamilton, L., Nature 175, 834 (1955).
Hashizume, H. and Imahori, K., J. Biochem. (Tokyo) 61, 738 (1967).
Langridge, R., Seeds, W., Wilson, H., Hooper, C., Wilkins, M., and Hamilton, L., J. Biophys. Biochem. Cytol. 3, 767 (1957).
Samejima, T. and Yang, J. T., Biochemistry 3, 613 (1964).
Samejima, T. and Yang, J. T., J. Biol. Chem. 240, 2094 (1965).
Samejima, T., Hashizume, H., Imahori, K., Fujii, I., and Miura, K., J. Mol. Biol. 34, 39 (1968).
Sarkar, P. K. and Yang, J. T., J. Biol. Chem. 240, 2088 (1965a).
Sarkar, P. K. and Yang, J. T., Biochemistry 4, 1238 (1965b).
Sarkar, P. K. and Yang, J. T., Arch. Biochem. Biophys. 112, 512 (1965c).

Sarkar, P. K. and Yang, J. T., in "Conformation of Biopolymers" (G. N. Ramachandran, ed.), Vol. I, p. 197, Academic Press, New York, 1967.
Sarkar, P. K., Wells, B., and Yang, J. T., J. Mol. Biol. 25, 563 (1967).
Sato, T., Kyogoku, K., Higuchi, S., Mitsui, Y., Iitaka, Y., Tsuboi, M., and Miura, K., J. Mol. Biol. 16, 180 (1966).
Tinoco, I., Jr., Woody, R. W., and Bradley, D. F., J. Chem. Phys. 38, 1317 (1963).
Ts'o, P. O. P., Rapaport, S. A., and Bollum, F. J., Biochemistry 5, 4153 (1966).
Tsuboi, M. and Higuchi, S., Proteins, Nucleic Acids, Enzymes (in Japanese) 13, 533 (1968).
Wilkins, M., Cellular Biology, Nucleic Acids and Virus, N. Y. Acad. Sci., 1957, p. 180.
Yang, J. T., Samejima, T., and Sarkar, P. K., Biopolymers 4, 623 (1966).